THE BIOLOGY OF THE SIERRA LUMINOUS MILLIPEDE, LUMINODESMUS SEQUOIAE, LOOMIS AND DAVENPORT

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In May of 1949 a group of students on an ecology field trip to the Sequoia National Forest (Camp Nelson, Tulare Co., California) brought back to the senior author a large number of striking and brilliantly luminescent millipedes. These animals were sent to Dr. E. Newton Harvey of Princeton University, who in turn forwarded them for identification to Mr. H. F. Loomis of the U. S. Plant Introduction Garden, Coconut Grove, Florida. Mr. Loomis found them to constitute an undescribed genus and species, and there resulted a collaborative original description (Loomis and Davenport, 1951). Reports of luminous millipedes are few. Bruner (1891) observed and described animals in Nebraska which were later given the name *Fontaria luminosa* by Kenyon (1893) and thus placed in the family Xystodesmidae, in which Luminodesmus belongs. It would appear, however, that these studies constitute the first investigations to be made of the life-history and physiology of luminescence of a luminous diplopod.

Luminodesmus sequoiae is a large and handsome millipede, the adults averaging 40 mm. in length and 8 mm. in width, of a pale tan or salmon color in late adult life, with a dark mid-dorsal line. In darkness the entire animal and its appendages give off a brilliant greenish-white glow, plainly visible to the dark-adapted eye at a considerable distance. Figure 1A is a photograph in ordinary (stroboscopic) illumination, while Figure 1B shows the animal taken in its own light only.

After discovery and description of the millipede, it became apparent that a thorough investigation of its ecology, distribution, life-history and physiology should be undertaken. Accordingly, in May of 1951 a trip was made to the type-locality for the purpose of collecting numbers of living animals, and some four hundred or more were brought back to Santa Barbara.

In the laboratory the millipedes proved easy to culture. A minimum of care was necessary to keep them alive and to raise the early stages. Adults were kept in a series of standard glass terraria filled to a depth of 5 or 6 inches with the rich, dark humus collected in their habitat. These terraria were covered with glass sheets and sealed with vaseline; about every 10 days they were opened and the humus

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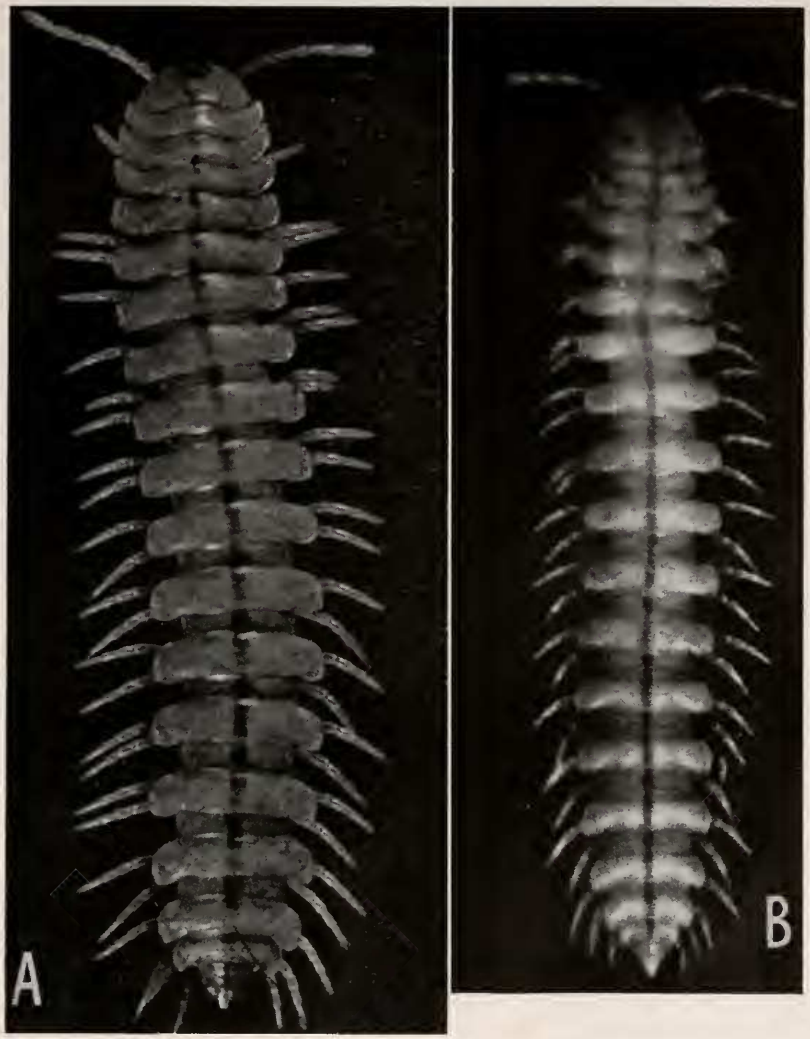

FIGURE 1. A. Adult *Luminodesmus sequoiae*. Approx. × 3. B. An adult *L. sequoiae* photographed in its own light. Animal curarized. Approx. × 3.

lightly sprinkled with water. Occasionally, when animals were removed for experimental purposes or a search for early stages was made, fresh humus was added from storage containers brought in from the field at the time the animals were collected.

ECOLOGY AND DISTRIBUTION

Luminodesmus sequoiae appears to have a very limited geographic range. So far the only records are one from the type-locality and an uncertain one from

Kaweah, Tulare Co. (Loomis and Davenport, 1951). During the summer of 1951 a brief visit was made to Yosemite, Kings Canyon and Sequoia National Parks during which it was found that no such animals had ever been observed in the parks to the knowledge of the Park Naturalists and their staffs. One ranger stated that he had observed "large luminescent animals" on a trail along the Merced River at low altitudes in Yosemite, but that these were not identified.

It appears probable that the animals may be limited to a particular environment with the characteristics of the type-locality, relatively moist with a thick, dark humus under a leafy litter made up of the disintegrating leaves of *Quercus, Acer,* etc., with an admixture of conifer needles. They must occur rarely if at all in the heavy coniferous forests of higher altitudes in the Sierra, characteristic of Kings Canyon and Sequoia National Parks; otherwise it would seem that they could scarcely have escaped notice. However, it is likely that further investigation of areas similar ecologically to the type-locality at lower altitudes within the Parks and in the foothills beyond their western limits may extend the range of Luminodesmus.

As has been stated, at the type-locality the animals may be so numerous on the surface that at night, when their bright patch of light attracts the eye, dozens can soon be collected. An effort was made in May of 1951 by Dr. E. R. Noble of the collecting group to determine how common the developmental stages might be in the upper layers of humus and soil. It was found that their distribution was extremely irregular. Several areas two feet square were carefully sorted over to a depth of 6 inches. In one such area 54 animals, ranging in length from 2–35 mm., were collected. Some areas of similar size had a far smaller population and in others no millipedes whatever could be found. No very striking differences in the characteristics of these areas could be observed which might account for the difference in distribution. It is apparent that the animals may be numerous enough locally so that their feeding activities could have a marked effect on the characteristics of the humus in their particular niche.

An investigation at the type-locality in October of 1951 showed that at this late season after a summer of extreme dryness adults were almost totally absent. A careful search on two consecutive nights revealed no adults at the surface. During daylight a number of areas where large numbers had been taken the previous spring were carefully investigated by digging to a depth of approximately 9 inches. It was found that although certain of these areas, particularly under the overhanging edges of logs and boulders, were filled with well defined workings and broken cocoons, no adults of the preceding summer were present. In some areas, however, numbers of the last four larval stages were discovered, seemingly distributed at random in the first 6 inches of soil, while a number of recently moulted adults were also present. All living animals were very inactive and there were indications of a high mortality due to desiccation, since a number of intact cocoons containing the dried remains of animals were found.

It would appear, therefore, that very few adult animals survive the long period of desiccation from mid-summer until the beginning of the winter rains, at least in years of light rainfall. This observation is borne out by the comment of one of the district foresters, stationed at Camp Nelson, who on being questioned, said that he was familiar with the animal and that it was a creature of the spring rains, disappearing at the beginning of the summer drought.

Life-History

Millipedes that were kept in humus in the sealed terraria in the laboratory thrived for many months, burrowing through the humus in their feeding and coming actively to the surface at night to wander about. The room in which they were kept varied in temperature in the usual way during the day from approximately 65° F. to 85° F. Because of the relatively constant temperature of this artificial environment and the knowledge that at least during several months of their development in nature they are subject to daily extremes of temperature from well below freezing to above 80° F., it did not seem worthwhile to go to the difficulty of determining in more than a general way the time-duration of each stage.

When the adult animals were first brought into the laboratory in May they were placed in fresh humus known to be free of eggs or early stages. Although mating was not observed in the laboratory, it is reasonable to assume that it freely occurred, since members of both sexes were placed in the terraria at random. The terraria were first investigated for the presence of early stages on July 19th and numbers of egg-masses were found (Fig. 2A). The date of laying was not known. These egg-masses varied widely in the number of contained eggs; three that were counted contained approximately 160, 70 and 165 respectively. The masses were laid in

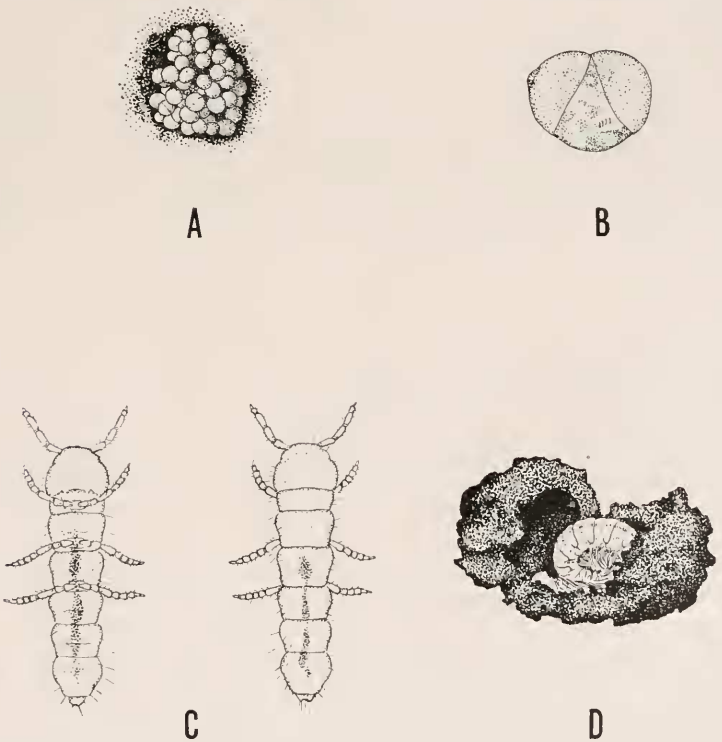

Figure 2. A. Egg-mass. Approx. × 2½. B. "Split-stage" egg. Approx. × 17. C. First instar larva in ventral and dorsal view. Approx. × 20. D. Opened cocoon of moulting larva. Approx. × 1¼.

loose cells of humus or in the closed ends of tunnel branches, a few inches below the surface. No firmly cemented cocoons of the type manufactured by moulting larvae appear to be constructed for these nests.

The eggs were approximately round, averaged 0.7 mm. in diameter, and appeared pearly, with a smooth surface. They were coated with a sticky substance that caused them to clump together, as has been described in other millipedes (Miley, 1927; Causey, 1943). As the eggs advanced in age the outer envelope took on a light brownish tinge.

In a number of egg clusters investigated, development had proceeded to a point which we have called the "split-stage" (Fig. 2B). In this stage, in which two halves of the split shell have been sprung apart like hinged hemispheres, there is contained a sac-like structure through which the developing larva with its appendages, etc., can just be perceived. Such a stage has been described for the juloid millipede, Arctobolus (Loomis, 1933) but not for the more closely related Polydesmid, Orthomorpha (Causey, 1943).

Loomis relates that in Arctobolus the first stage after hatching consists of a grub-like creature without appendages contained in a delicate semi-transparent membrane. We believe, although it could not be demonstrated, that in Luminodesmus this stage is passed briefly while in the "split-stage." When true hatching occurs, typical six-legged first instar larvae appear. In a large nest of "split-stage" eggs, first instar larvae were observed hatching from the membranous structure within the halves of the shell.

That the duration of time in the egg is not less than two weeks was demonstrated, since first instar larvae hatched from July 31st to August 3rd from "split-stage" eggs that had been isolated in small glass jars on July 19th.

No luminosity whatever could be observed in large egg-masses either before or in the "split-stage." All observations on luminescence were made in a dark-room after 15–20 minutes of dark-adaptation by means of Polaroid Corp. type-XDA8FAP red lenses. Single first instar larvae were checked for luminescence and none could be observed. An opportunity to check larvae hatching in numbers from a clump of eggs showed that enough luminescence is present in first instar larvae for it to be just apparent to the scanning, dark-adapted eye when the larvae are crowded together in a mass. Under these circumstances there is just enough luminosity present to be picked up with rod-vision in the thoroughly dark-adapted eye.

Whether luminosity is present in the egg itself has not been definitely determined. It is certainly not evident even when the eggs are in large masses; it is possible, however, that the opacity of the envelope prevents passage of light to the exterior.

The first instar larva may be seen in ventral and dorsal view in Figure 2C. This stage has 7 post-cephalic segments and averages 1.7 mm. in length. The animals are very active on hatching and move about feeding on the humus. At hatching they are transparently pale white, but shortly after commencing feeding, the gut, filled with black humus, becomes very evident.

A number of first instar larvae were isolated in humus on July 19th. By August 10th numbers of these were observed to have constructed the typical cemented cocoons in which moulting of larvae occurs. A cocoon broken open with a late larva *in situ* is shown in Figure 2D. The cocoon closely resembled that described

by Miley (1927) in Euryurus. Cocoons are ball-like, smooth on the inside and rough on the exterior, of a dark, smoothly homogenous consistency apparently the result of the working together of humus and earth with some secretion. These structures are entirely closed and some possess the chimney-like extensions described by Miley. It is not unusual to find more than one individual in a single cocoon, particularly in some of the early moulting stages. As other authors have described, the millipedes are extremely inactive when in the process of moulting in the cocoons. That there is a general slowing down in metabolism at this time is indicated by the marked decrease in luminosity. No animals have been observed to lose their luminosity at any time from larva to adult, but during moulting the intensity is greatly diminished.

On August 20th the same isolation jar was examined, and a number of second instar larvae had appeared and were actively feeding. The time duration in the first instar was therefore approximately 33 days. Second instar larvae had 6 pairs

TABLE I

Characteristics of developmental stages

	Pairs of legs		No. post-cephalic segments	Average length in mm.
	♂	♀		
1st stage	3	3	7	1.7
2nd stage	6	6	9	2.9
3rd stage	11	11	12	3.8
4th stage	16	17	15	6.5
5th stage	22	23	17	8.0
6th stage	26	27	18	13.4
7th stage	28	29	19	22.1
Adult	30	31	20	41.0

of appendages, 9 post-cephalic segments and averaged 2.9 mm. in length. Total luminosity of these animals had increased so much that the position of three animals in a Petri dish could clearly be observed after 10 minutes dark-adaptation.

On September 9th these second stage larvae were observed to have constructed cocoons and on September 20th a number of third stage larvae had appeared. These possessed 11 pairs of legs, 12 post-cephalic segments and averaged 3.8 mm. in length. The time duration of the second instar was approximately 31 days.

Subsequent moults appear to proceed in much the same way, although as this is written laboratory-raised larvae have only reached the fourth larval stage. Fifth, sixth and seventh stage larvae were collected in the field in May and October of 1951.

The characteristics of the developmental stages are summarized in Table I.

Gonopod development in the males of *Luminodesmus sequoiae* resembles that described for other polydesmoid millipedes. In the third larval stage no sexual dimorphism can be observed in the appendages. In the fourth larval stage, in place of the anterior pair of legs of the 7th post-cephalic segment there appear two very small raised domes (Fig. 3A). In the fifth larval stage these have become

slightly larger. In the sixth and seventh larval stages proximal and distal segments are plainly apparent, the former segment being sunk into a pit in the sternite (Fig. 3B). The fully developed gonopod appears in the adult (Fig. 3C).

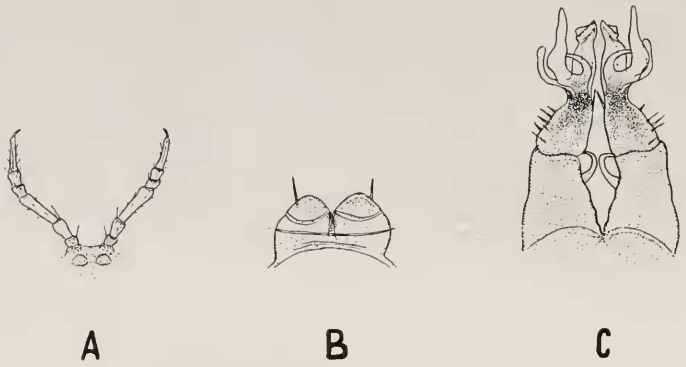

FIGURE 3. A. Appendages of the 7th post-cephalic segment in male fourth larval stage, showing gonopods appearing in position of eighth leg-pair. B. Developing gonopods of the seventh instar. C. Gonopods of adult male. All approx. × 13.

THE SOURCE AND NATURE OF LUMINESCENCE

While an extensive study of the luminescence of *Luminodesmus sequoiae* has yet to be made, some observations of general interest can be presented at this time.

The luminescence is continuous and appears to be under no voluntary control. During periods of general inactivity (*e.g.*, moulting) a decrease in intensity may occur. Momentary variations in light intensity in the entire animal may occur, but at the present time it cannot be said with certainty that these changes are correlated with changes in bodily activity. It has often been noted that luminosity may fade within segments or groups of segments under abnormal conditions (such as preceding death from local damage, poisons, CO_2).

Several observers have stated that they had an impression of a particulate light source when they examined the brilliant dorsal integument. Figure 4, an enlargement of five segments taken in their own light, strengthens the impression that there are in the integument scattered points of more intense luminosity. It is possible, however, that this effect may result from the breaking up of uniform light coming from deeper layers by the surface sculpture of the integument. This photograph, as well as Figure 1B, was taken in complete darkness with a Leica f-2 on Super-XX panchromatic film. The exposure time was one hour. Animals were inactivated by injection of 0.25 cc. *d*-tubocurarine chloride at a concentration of 3 mg./cc. The neuro-muscular blocking agent appeared to have no direct effect on luminescence.

Efforts to determine the exact source of the luminescence have been made as follows. Dissection and observations have determined that the fat bodies, viscera, tracheae, musculature and blood are not luminous. Frozen sections of adult animals have been made with CO_2. If these are rapidly thawed out under a jet of oxygen and observed in darkness, it can be seen that the source of the luminescence lies in the integument, since a cross-sectional "pipe" of light can be observed. The

light from such sections fades out in a few minutes and is so pale and so diffuse as to make it impossible to resolve the source under the microscope. If the sclerotized integument of the animal is scraped with a scalpel on the outside so that flakes are removed, no trace of luminosity can be observed in these flakes. Further evidence that the source of the luminescence may lie in the deeper integumentary layers, perhaps the epidermis or endocuticle, is presented by the fact that the antennae and legs, containing non-luminous blood, muscle, etc., are brilliantly luminescent. Under the dissecting microscope in darkness, appendages appear to give a relatively uniform glow with the intensity appearing greater to the eye at the curving surfaces at the margins, where there is, of course, greater integumentary thickness.

Paraffin sections of adult animals and fourth stage larvae have been cut and stained with Mallory's triple stain with Orange G. It has so far been impossible to discern either in the endocuticle, which is traversed by numerous canals, or the epidermis, the granules which have so often been described in animal cells that are

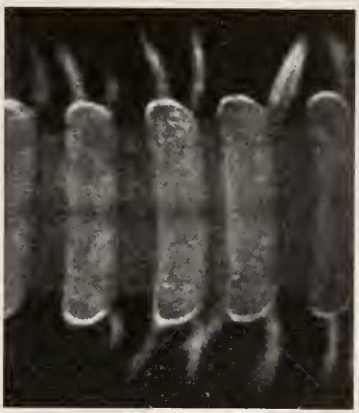

FIGURE 4. A dorsal view taken in animal's own light showing particulate appearance of light source and brilliance of lateral margins. Animal curarized. Approx. × 4.

auto-luminescent. Fresh, unstained smears of integument or of appendages, or smears stained with methylene blue have given no indication under oil-immersion of the presence of such bodies or of bacteria which might be suspected of being the source of luminescence.

Efforts to make extracts of Luminodesmus which would retain luminescence *in vitro* have so far been without success. Extracts prepared by grinding whole or eviscerated animals in water and filtering the extract into an evaporating dish glowed very briefly and went out. To date it has not been possible to re-activate the extract by adding various combinations of Photinus extract (including concentrated "luciferase" extracts made from freshly dried lanterns), magnesium ion and adenosine triphosphate (*cf.* McElroy and Harvey, 1951, for further references). Attempts to show classical "luciferin-luciferase" activity by combining heat-treated fractions with non-heat-treated ones (Harvey, 1940) have not been successful. However, it should be possible to study the effects of some factors on the emission of light in Luminodesmus in a semi-isolated system, since it has recently been found

that single legs and antennae if placed in distilled water will retain full luminosity for at least four hours and overnight if refrigerated.

As has been stated (Loomis and Davenport, 1951), if the animal is irradiated with an ultra-violet light source, a brilliant chartreuse-green fluorescence appears. This fluorescence comes from the entire integument and often most brilliantly from areas which are not as intensely luminescent as others, such as the ventral surface. Blood, viscera and trachae are not fluorescent. That a yellow-green fluorescent compound is directly involved in the luminescence of fire-flies has been demonstrated (Strehler and McElroy, 1949). However, it should be borne in mind that many fluorescent compounds are known that are not concerned with the luminescent reaction.

That Luminodesmus, like most other luminous forms, must have oxygen in order to maintain full intensity of light production, was demonstrated by the use of nitrogen and carbon dioxide. Individual animals were placed in the bottom of a cotton-stoppered U-tube through which gases were drawn. The luminescence of animals in an atmosphere of pure nitrogen rapidly faded so that after five minutes only a dim glow was perceptible. This minimum light production was maintained as long as animals were observed (three hours). During this time the animals were quiescent, but apparently the effect on luminescence was specific, for the introduction of oxygen to the U-tube immediately restored full and brilliant light, while the animals returned to normal, unimpaired activity. Whether or not the dim light given off during prolonged exposure to nitrogen also requires small amounts of molecular oxygen remains to be investigated (*cf.* Harvey, 1940, p. 127).

Atmospheres of pure CO_2 were found to cause the rapid and complete disappearance of luminescence within two minutes and also to be extremely toxic. Animals exposed to the gas for 15 minutes not only failed to luminesce but were completely inactivated. Re-introduction of oxygen to the U-tube caused a partial return of light production almost immediately, but that this exposure to CO_2 was close to the lethal point was demonstrated by the fact that some animals failed to recover complete luminosity and activity, and ultimately died. Whether or not CO_2 has a specific effect on the luminescent system is yet to be demonstrated.

Animals were subjected to HCN in a collecting tube strong enough to kill Hymenoptera (Apis) almost instantaneously. The millipedes showed a marked decrease in activity at the end of an hour but no decrease in luminescence below the normal brilliant level. At the end of approximately three hours the animals were completely inactive (dead?) and the luminescence greatly reduced. Neither activity nor full luminescence ever returned when these animals were placed in an atmosphere of pure oxygen. These observations suggest that cyanide is relatively non-toxic to the luminescent system and does not affect it *in vivo* until the lethal point is reached. This is of interest in view of the demonstration of McElroy and Strehler (1949) that HCN not only fails to inhibit luminescence in luminous bacteria and in isolated biochemical systems but actually stimulates light production.

Finally it may be asked what the function of the luminescence is in Luminodesmus. It would appear that it cannot serve as a recognition factor, since all members of this millipede order are blind. As will be seen, Luminodesmus, like many other millipedes, gives off an offensive odor. Cook (1900) says "this absence of eyes also renders apparently meaningless the fact recorded by Bruner and Kenyon that

the repugnatorial secretion of a Nebraska species" (*Fontaria luminosa* Kenyon) "is luminous . . . but when the nocturnal habits of the animals are considered, phosphorescence may be looked upon as affording a protection additional to that of the odor of the repugnatorial fluid."

This hypothesis that a "warning luminescence" has evolved in these millipedes can only be proved by the most carefully controlled field experiments involving nocturnal predators; at the present time it must be said that the role the light plays in the life of the animals is problematical.

Release of HCN

The release of HCN by millipedes has been noted by a number of authors in many species (Guldensteeden-Egeling, 1882; Verhoeff, 1928; etc.).

When collecting live Luminodesmus at the type locality and many times thereafter in handling experimental animals, observers have noted a powerful "cyanide jar" smell. It seemed advisable, therefore, to test for the presence of cyanide. Accordingly, the highly specific and delicate test developed by Fox (1934) was employed. Air is drawn through a system to be tested and then bubbled through an alkaline silver iodide suspension. If HCN is present, the KCN formed immediately dissolves the AgI, the cloudy bluish suspension disappears, and the resulting solution is perfectly clear.

If air was drawn over soil in which the millipedes had been living, a negative test resulted, but air drawn over a single animal which had been slightly agitated in a test-tube gave a strong positive reaction, indicating the release by the animal of HCN.

A biological test demonstrated the poisonous effects of the material released by the millipedes. Bees (Apis) were enclosed in test-tubes with millipedes and slightly agitated. Within three minutes the bees completely lost motor control and if enclosed thus for more than 15 minutes failed to recover. Control bees showed no diminution of angry activity if enclosed in similar stoppered tubes for half an hour.

No direct physiological connection between the phenomenon of luminescence and the release of HCN in Luminodesmus has been demonstrated. Kenyon (1893) and Cook (1900) discuss the possibility that in *F. luminosa* the repugnatorial glands are also the source of the "bead-like" luminosity. It would be of great interest to determine whether the glands of this animal, like those of Luminodesmus and other polydesmoids, release HCN and whether they are in fact involved in the luminescence, particularly in view of the demonstration of McElroy and Strehler, cited above, that cyanide stimulates the light-emitting reaction in other organisms.

Summary

1. The characteristics, distribution and habitat of the millipede, *Luminodesmus sequoiae* Loomis and Davenport are discussed. The life-history and the development of the male gonopods are described.

2. Luminescence is continuous, under no voluntary control, and first appears on hatching; its source apparently lies in the deeper layers of the integument.

3. Extracts of Luminodesmus glow briefly and go out. Experiments to elicit a light flash from such extracts with combinations of Photinus lanterns, magnesium

ion and adenosine triphosphate, and to demonstrate classical "luciferin-luciferase" activity have been without success. The integument of Luminodesmus gives a brilliant yellow-green fluorescence in ultra-violet light.

4. Nitrogen atmospheres reversibly extinguish luminescence, carbon dioxide irreversibly so, while cyanide has little effect on luminescence until the lethal point is reached. Chemical and biological tests have indicated that Luminodesmus, like other millipedes, releases HCN.

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